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# Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test

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## Introduction

The photosynthetic apparatus, and especially photosystem II (PSII), is well known to be very sensitive to different stresses. Stress and stress adaptation can therefore be monitored by following the behaviour of the photosynthetic apparatus. Chlorophyll (Chl) *a* fluorescence, though corresponding to a very small fraction of the dissipated energy from the photosynthetic apparatus, has been proven to be a very useful, non-invasive tool for the investigation of its structure and function. At ambient temperature Chl *a* fluorescence is basically emitted by PSII. The fluorescence transient, known as the Kautsky transient, consists of a rise completed in less than 1 s and a subsequent slower decline towards a steady state. The rise reflects the accumulation of the reduced form of the primary quinone acceptor  $Q_A$ , otherwise the closure of the reaction centres (RCs), which is the net result of  $Q_A$  reduction due to PSII activity and  $Q_A$ -reoxidation due to photosystem I (PSI) activity. When the photosynthetic sample is kept for few minutes in the dark,  $Q_A$  is fully oxidised, hence the RCs are all open, and the fluorescence yield at the onset of illumination is denoted as  $F_0$ . The maximum yield  $F_p$  at the end of the fast rise, depending on the achieved reduction-oxidation balance, acquires its maximum possible value - denoted as  $F_M$  - if the illumination is strong enough to ensure the closure of all RCs. A lot of information has been driven during the last sixty years from the fluorescence transient (see e.g. Krause and Weis 1991; Govindjee 1995).

Transients recorded with high time-resolution fluorimeters, e.g. with the PEA-instrument (data acquisition every 10  $\mu$ s for the first 2 ms and 1 ms thereafter), have provided additional and/or more accurate information (Strasser and Govindjee 1992; Strasser et al. 1995). It was shown that the fluorescence rise kinetics is polyphasic exhibiting clearly, when plotted on a logarithmic time scale, the steps J (at 2 ms) and I (30 ms) between the initial O ( $F_0$ ) and maximum P level ( $F_p$ ); moreover, a much more precise detection of  $F_0$ , as well of the initial slope which offers a link to the maximum rate of photochemical reaction, is succeeded.

All oxygenic photosynthetic material investigated so far using this method show this polyphasic rise, labelled as O-J-I-P. The shape of the O-J-I-P fluorescence transient has been found to be very sensitive to stress caused by changes in different environmental conditions, such as light intensity, temperature, drought, atmospheric  $CO_2$  or ozone elevation and chemical influences (see e.g. Srivastava and Strasser 1996, 1997; Tsimilli-Michael et al. 1996, 1999; Van Rensburg et al. 1996; Krüger et al. 1997; Ouzounidou et al. 1997; Clark et al. 1998, 2000).

A quantitative analysis of the O-J-I-P transient has been introduced (Strasser and Strasser 1995) and further developed, named as the "JIP-test" after the steps of the transient, by which several phenomenological and biophysical - structural and functional - parameters quantifying the PSII behaviour are calculated (for a review see Strasser et al. 2000). The JIP-test was proven to be a very useful tool for the *in vivo* investigation of the adaptive behaviour of the photosynthetic apparatus and, especially, of PSII to a wide variety and combination of stressors, as it translates the shape changes of the O-J-I-P transient to quantitative changes of the several parameters. Hence the deviation of the constellation of these parameters from that of the non-stressed condition expresses quantitatively evaluated strains, fingerprints of the stressors on the photosynthetic organism.

We here present an outline of the JIP-test, preceded by a summary of our stress concept, which is the basis of our approach. Moreover, we present an application of the JIP-test in a case study referring to the response of the photosynthetic apparatus in *Vicia faba* leaves upon the diurnal changes of the incident light intensity. With the same methodology, by which fingerprints of several kinds of stressors on the behaviour and performance of the photosynthetic apparatus have been so far detected, identified, analysed and "mapped" (Srivastava and Strasser 1996, 1997; Tsimilli-Michael et al. 1996, 1999, 2000; Krüger et al. 1997; Clark et al. 1998, 2000), the quantified behaviour of the photosynthetic apparatus can be used as a bio-indicator of any stress, from daily rhythm to global changes.

## The stress concept

The term "stress" comes from physics where it has been precisely defined, measured by the strain it provokes. However, concerning biology it has been given widely differing meanings. Probably due to an extension of the physical meaning, many of them converge in attributing stress to any environmental factor "unfavourable" for the living organism under consideration. Our approach is different in principle. It is focused on the dynamic character of the relation between organism and environment, keeping from the physical approach the concept of "action-reaction", and offers the possibility of analytical description and quantification (for further details on the Stress Concept see Strasser, 1985; 1988; Tsimilli-Michael et al., 1996; Tsimilli-Michael and Strasser, 2001).

We consider that stress has a relative meaning, with non-stress as the reference condition. More precisely, we consider stress as a deviation from the non-stress situation. The latter is not statically but dynamically defined: it is the situation at which the organism is in "harmony" with its environment, or equivalently, the plant is at its thermodynamic optimal state, which can be regarded, based on infe-

rences from open system thermodynamics, as the state of minimal entropy production. In other words, non-stress is defined as corresponding to thermodynamic optimality and stress to suboptimality. Accordingly, any change in the environmental input is a stressor in the sense that it disturbs the achieved optimality and leads the system into suboptimality. Because of the thermodynamic demand for optimality, suboptimality creates a force under which the system undergoes state changes, i.e. changes in its conformation/structure, seeking for a new optimality. The new optimal state is the attraction point for the state change walk and the force, defined as the state change force, depends on how far from the attraction point the suboptimal condition of the system is. If the system succeeds in reaching the attraction point, suboptimality becomes zero, the state change force vanishes and the new stability, i.e. harmony with the environment, is established. Stress adaptation is, thus, the sequence of processes which realise these state changes. If the deviation of the new, adapted, state from the former state becomes measurable, we call it a strain (for a schematic presentation see Figure 1). The "deformation" of the conformational/ structural parameters of a system may be elastic (reversible) or plastic (irreversible). The plasticity of a deformation of a certain parameter is revealed if the system, after been exposed to a cyclic environmental change, still exhibits a strain, termed as residual strain concerning this parameter.

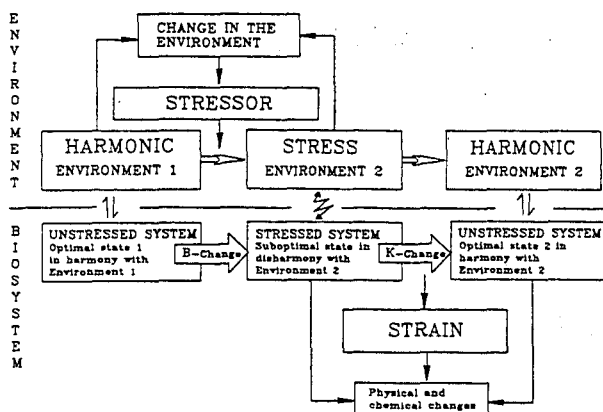


Figure 1. A schematic presentation of a state-change as a consequence of an applied stressor, demonstrating also the terms used in the stress concept and defined as: Stressor: every factor that provokes "stress"; Stress: every established condition which forces a system away from its thermodynamic "optimal state"; Optimal state of a biological system: the state at which the system is in full "harmony" with its environment; Harmony of a biological system with its environment: the achieved situation at which the system does not tend to change any activity or conformation; Strain: any physical or chemical change caused by stress. Moving within one state corresponds to changes of behaviour (B-change), leading to suboptimality. Moving from one state function to another corresponds to conformational changes (K-change) leading to optimality.

The environmental conditions never cease to manifest alterations and, thus, the system perpetually undergoes stress and concomitant stress adaptation processes, seeking and approaching harmony with its environment. In this concept no environmental factor is considered a priori as unfavourable and, consequently, the plant has not to resist, but it simply reacts. Depending on the physiological impact, the stress can be classified as a constructive or destructive stress (Larcher, 1987). As far as the system manages to adapt, which means that the attraction point is within realistic limits stress is not only harmless but even constructive because it results in improved resistance and adaptive evolution. If the attraction point is beyond the realistic limits, adaptation cannot succeed and, therefore, stress leads to damages or destruction. However, the realistic limits are different for different organisms. Moreover, the limits for a certain organism can vary if more stressors are combined (Srivastava and Strasser, 1996; Krüger et al., 1997).

The environmental changes can provoke quite different responses on an organism. It may exhibit a relative stability in its behaviour, i.e. homeostatic behaviour, or reveal even a wide variability. However, in both cases the organism does undergo a shift to suboptimality, being forced to increase the entropy production either to maintain the established state or to search a new one. Moreover, by a suitable deconvolution of the system's behaviour in structural/ conformational and functional parameters (see The JIP-test), it was found that, upon stress, the various parameters undergo modifications that differ concerning both their extent and their degree of elasticity, thus indicating that complex regulation mechanisms are employed to realise stress adaptation: the down regulation of some parameters serves for the maintenance of others (see e.g. Tsimilli-Michael et al., 1999, 2000).

Screening different plants for the same environmental changes one can observe that each species acquired specific survival strategies during evolution to respond to stress. Moreover, experimental data revealing the ability of photoprotection show that certain strains may as well carry the information of certain protective capabilities, such as high or low temperature resistance (hardening) or high light resistance (Srivastava and Strasser, 1996; 1997). This can be regarded as a memory of the stressors they had been exposed to, i.e. a memory about the past, influencing future behaviour. These phenomena could be correlated with signal transduction and gene induction mechanisms which have been acquired during evolution in an environment of periodically changing conditions.

### The JIP-test

Chl *a* fluorescence transients exhibited by any photosynthetic material are measured by a PEA fluorimeter (Plant Efficiency Analyser, built by Hansatech Instruments Ltd, King's Lynn Norfolk, PE 30 4NE, GB). The transients are induced by a red light (peak at 650 nm) of 600 W m<sup>-2</sup> (3200 μE m<sup>-2</sup> s<sup>-1</sup>) provided by an array of six light-emitting diodes, and recorded for 1 s with 12 bit resolution: the data acquisition is every 10 μs for the first 2 ms and every 1 ms thereafter (for details see Strasser et al. 1995).

A typical Chl *a* fluorescence transient O-J-I-P is shown in Figure 2, plotted on a logarithmic time scale so that the intermediate steps are clearly revealed. The following original data (see Figure 2) are utilised by the JIP-test: the maximal measured fluorescence intensity,  $F_P$ , equal here to  $F_M$  since the excitation intensity is high enough to ensure the closure of all RCs of PSII; the fluorescence intensity at 50  $\mu$ s considered as the intensity  $F_0$  when all RCs are open; the fluorescence intensity at 300  $\mu$ s ( $F_{300\mu s}$ ) or 150  $\mu$ s ( $F_{150\mu s}$ ) required for the calculation of the initial slope  $M_0 = (dV/dt)_0 \cong (\Delta V/\Delta t)_0$  of the relative variable fluorescence (*V*) kinetics (see insert in Figure 2 and Table 1); the fluorescence intensities at 2 ms (J step) denoted as  $F_J$ , and at 30 ms (I-step) denoted as  $F_I$  (for a review see Strasser et al. 2000).

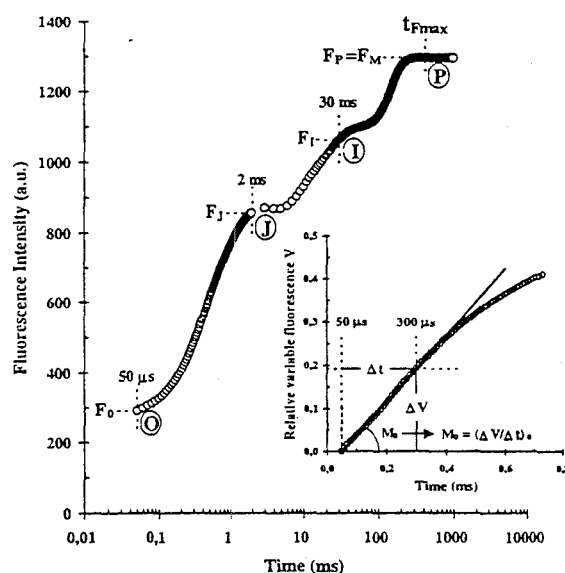


Figure 2. A typical Chl *a* polyphasic fluorescence rise O-J-I-P, exhibited by higher plants. The transient is plotted on a logarithmic time scale from 50  $\mu$ s to 1 s. The marks refer to the selected fluorescence data used by the JIP-test for the calculation of structural and functional parameters. The signals are: the fluorescence intensity  $F_0$  (at 50  $\mu$ s); the fluorescence intensities  $F_J$  (at 2 ms) and  $F_I$  (at 30 ms); the maximal fluorescence intensity,  $F_P = F_M$  (at  $t_{Fmax}$ ). The insert presents the transient expressed as the relative variable fluorescence  $V = (F - F_0)/(F_M - F_0)$  vs. time, from 50  $\mu$ s to 1 ms on a linear time scale, demonstrating how the initial slope, also used by the JIP-test, is calculated:  $M_0 = (dV/dt)_0 \cong (\Delta V/\Delta t)_0 = (V_{300\mu s})/(0.25 \text{ ms})$ .

The JIP-test refers to a translation, through the formulae of Table 1, of the original data to the following biophysical parameters, all referring to time zero (onset of fluorescence induction), that quantify the PSII behaviour: (a) the specific energy fluxes (per reaction centre) for absorption (ABS/RC), trapping ( $TR_0/RC$ ), dissipation ( $DI_0/RC$ ) and electron transport ( $ET_0/RC$ ); (b) the flux ratios or yields, i.e. the maximum quantum yield of primary photochemistry ( $\Phi_{P_0} = TR_0/ABS$ ), the efficiency ( $\Psi_0 = ET_0/TR_0$ ) with which a trapped exciton can move an electron into the electron transport chain further than  $Q_A^-$  and the quantum yield of electron transport  $\Phi_{E_0} = ET_0/ABS = \Phi_{P_0} \cdot \Psi_0$ ; the pheno-

menological energy fluxes (per excited cross section, CS) for absorption (ABS/CS), trapping ( $TR_0/CS$ ), dissipation ( $DI_0/CS$ ) and electron transport ( $ET_0/CS$ ). The concentration of active PSII reaction centres per excited cross section (RC/CS) is also calculated. The set of formulae used for this translation is presented in Table 1.

Extracted and technical fluorescence parameters		
$F_0$	=	$F_{50\mu s}$ , fluorescence intensity at 50 $\mu$ s
$F_{150}$	=	fluorescence intensity at 150 $\mu$ s
$F_{300}$	=	fluorescence intensity at 300 $\mu$ s
$F_J$	=	fluorescence intensity at the J-step (at 2 ms)
$F_M$	=	maximal fluorescence intensity
$t_{Fmax}$	=	time to reach $F_M$ , in ms
$V_J$	=	$(F_{2ms} - F_0)/(F_M - F_0)$
Area	=	area between fluorescence curve and $F_M$
$(dV/dt)_0$ or $M_0$	=	$4 \cdot (F_{300} - F_0)/(F_M - F_0)$
$S_m$	=	Area / $(F_M - F_0)$
$B_{FV}$	=	$1 - (S_m / t_{Fmax})$
$N$	=	$S_m \cdot M_0 \cdot (1/V_J)$ turn over number of $Q_A$
Quantum efficiencies or flux ratios		
$\Phi_{P_0}$ or $TR_0/ABS$	=	$1 - (F_0/F_M)$ or $F_V/F_M$
$\Phi_{E_0}$ or $ET_0/ABS$	=	$[1 - (F_0/F_M)] \cdot \Psi_0$
$\Psi_0$ or $ET_0/TR_0$	=	$1 - V_J$
Specific fluxes or specific activities		
ABS/RC	=	$M_0 \cdot (1/V_J) \cdot (1/\Phi_{P_0})$
$TR_0/RC$	=	$M_0 \cdot (1/V_J)$
$ET_0/RC$	=	$M_0 \cdot (1/V_J) \cdot \Psi_0$
$DI_0/RC$	=	$(ABS/RC) - (TR_0/RC)$
Phenomenological fluxes or phenomenological activities		
ABS/ $CS_0$	=	$F_0$ or an other useful expression *
$TR_0/CS_0$	=	$\Phi_{P_0} \cdot (ABS/CS_0)$
$ET_0/CS_0$	=	$\Phi_{P_0} \cdot \Psi_0 \cdot (ABS/CS_0)$
$DI_0/CS_0$	=	$(ABS/CS_0) - (TR_0/CS_0)$
Density of reaction centres		
RC/ $CS_0$	=	$\Phi_{P_0} \cdot (V_J/M_0) \cdot F_0$ *
Performance index		
$PI_{ABS}$	=	$(RC/ABS) \cdot [\Phi_{P_0}/(1-\Phi_{P_0})] \cdot [\Psi_0/(1-\Psi_0)]$
Driving forces		
$DF_{ABS}$	=	$\log [PI_{ABS}]$
$DF_{RC}$	=	$\log [RC/ABS]$
$DF_{\Phi}$	=	$\log [\Phi_{P_0}/(1-\Phi_{P_0})]$
$DF_{\Psi}$	=	$\log [\Psi_0/(1-\Psi_0)]$
* when expressed per $CS_M$ , $F_0$ is replaced by $F_M$		

Table 1. Summary of the JIP-test formulae using data extracted from the fast fluorescence transient O-J-I-P

Recently the performance index PI was introduced (Strasser et al. 1999; Srivastava et al. 1999; Tsimilli-Michael et al. 2000; for a review see Strasser et al. 2000). Here we present the performance index on absorption basis,  $PI_{ABS}$ :

$$PI_{ABS} = \frac{\gamma_{RC}}{1-\gamma_{RC}} \cdot \frac{\Phi_{P_0}}{1-\Phi_{P_0}} \cdot \frac{\Psi_0}{1-\Psi_0} = \frac{RC}{ABS} \cdot \frac{\Phi_{P_0}}{1-\Phi_{P_0}} \cdot \frac{\Psi_0}{1-\Psi_0}$$

where  $\gamma_{RC}$  is the fraction of reaction centre chlorophylls relatively to the total chlorophyll:  $\gamma_{RC} = Chl_{RC}/Chl_{total}$ . Since  $Chl_{total} = Chl_{antenna} + Chl_{RC}$ , we get:  $\gamma_{RC}/(1-\gamma_{RC}) = Chl_{RC}/Chl_{antenna} = RC/ABS$ .

Substitution of the biophysical by the experimental parameters (see Table 1) results in:

$$PI_{ABS} = \frac{1-(F_0/T_M)}{M_0/V_J} \cdot \frac{F_M-F_0}{F_0} \cdot \frac{1-V_J}{V_J}$$

As defined, the performance index is a product of expressions of the form  $[p_i/(1-p_i)]$ , where the  $p_i$  ( $i=1, 2, \dots, n$ ) stand for probabilities or fractions. Such expressions are well-known in chemistry, with  $p_i$  representing e.g. the fraction of the reduced and  $(1-p_i)$  the fraction of the oxidised form of a compound, in which case  $\log[p_i/(1-p_i)]$  expresses the potential or driving force for the corresponding oxidation-reduction reaction (Nernst's equation). Extrapolating this inference from chemistry, the  $\log[PI_{ABS}]$  can be defined as the driving force ( $DF_{ABS}$ ) for photosynthesis of the observed system, created by summing the partial driving forces for each of the several energy bifurcations (all at the onset of the fluorescence rise O-J-I-P):

$$DF_{ABS} = \log(PI_{ABS}) = \log \left[ \frac{RC}{ABS} \right] + \log \left[ \frac{\varphi_{P_0}}{1-\varphi_{P_0}} \right] + \log \left[ \frac{\psi_0}{1-\psi_0} \right]$$

Introducing the notations  $DF_{RC} = \log[RC/ABS]$ ,  $DF_{\varphi} = \log[\varphi_{P_0}/(1-\varphi_{P_0})]$  and  $DF_{\psi} = \log[\psi_0/(1-\psi_0)]$ , we can write the above equation as  $DF_{ABS} = DF_{RC} + DF_{\varphi} + DF_{\psi}$ .

It is worth pointing out that the JIP-test reveals changes in the PSII behaviour that cannot be detected by the commonly used  $\varphi_{P_0} = (F_M - F_0)/F_M$ , which is the least sensitive of all parameters. Moreover,  $\varphi_{E_0}$  and  $PI$  are related to the productivity of photosynthetic metabolites and, hence, they offer a diagnostic tool for the biomass production capability.

The JIP-test, developed and tested both in the laboratory and in several applications, is well accepted to provide a detection, description and quantification of the dynamic capacities of the photosynthetic sample, as they are expressed by behaviour patterns provoked by stress. It has been widely and successfully used for the investigation of PSII behaviour in various photosynthetic organisms under different stress conditions, which result in the establishment of different physiological states, as well as for the study of synergistic and antagonistic effects of different co-stressors. Moreover, it has been proven a useful tool for the investigation of the beneficial effects of the participants in rhizosphere/mycorrhizosphere systems on the plant, as well as of their interactions.

The big advantages of the method are: (1) it provides an early diagnosis of primary stress effects on the photosynthetic organisms; (2) it is rapid – less than a few seconds are needed for each measurement; (3) it can be applied in vivo; (4) it can be carried out anywhere – in the field, in the green house or even in tissue cultures – and even on samples as small as 2 mm<sup>2</sup>; (5) it is non-invasive; (6) it is very inexpensive.

#### A case study: Response to diurnal light intensity changes

A case study is presented as an example of the JIP-test application. This study refers to the response of the photosynthetic apparatus in *Vicia faba* leaves upon the diurnal changes of the incident light intensity, from dawn (6:30 am) to dusk (9:30 pm). The extracted data (see Table 2) from each of the fluorescence transients recorded every 1.5 h

from 6:30 to 21:30 were used to calculate the whole set of the parameters, according to the formulae shown in Table 1. The set of results from three measurements, namely at 6:30 (incident light intensity  $I_0 = 4 \mu E m^{-2} s^{-1}$ ), 13:30 ( $I_0 = 550 \mu E m^{-2} s^{-1}$ ) and 21:30 ( $I_0 = 0$ ) are presented in Table 3. Numbers in parentheses express the normalized values

Time	6:30 am	1:30 pm	9:30 pm
Light intensity ( $\mu E m^{-2} s^{-1}$ )	4	550	0
<b>F extremes</b>			
$F_0$	620 (1)	610 (0.98)	612 (0.99)
$F_M$	3229 (1)	2033 (0.63)	2963 (0.92)
$F_V / F_0$	4.21 (1)	2.33 (0.55)	3.84 (0.91)
<b>F dynamics</b>			
$V_J$	0.56 (1)	0.70 (1.24)	0.57 (1.01)
$V_I$	0.81 (1)	0.66 (0.81)	0.80 (0.98)
$(dV/dt)_0$	0.82 (1)	1.37 (1.67)	1.00 (1.22)
<b>AREAS</b>			
$S_m$	25.33 (1)	19.42 (0.77)	17.87 (0.71)
$S_m / t_{fmax}$	0.090 (1)	0.067 (0.75)	0.094 (1.05)
$N$	36.78 (1)	38.07 (1.03)	31.20 (0.85)

Table 2. The values of extracted and technical fluorescence parameters directly obtained from the recorded fluorescence transients. Numbers in parentheses express the normalized values over the corresponding values at 6:30 am (control)

Time	6:30 am	1:30 pm	9:30 pm
Light Intensity ( $\mu E m^{-2} s^{-1}$ )	4	550	0
<b>FLUXES - ACTIVITIES per RC</b>			
$ABS/RC$	1.80 (1)	2.80 (1.56)	2.20 (1.22)
$TR_0/RC$	1.45 (1)	1.96 (1.35)	1.75 (1.20)
$ET_0/RC$	0.63 (1)	0.59 (0.93)	0.75 (1.18)
<b>FLUXES RATIOS = YIELDS</b>			
$TR_0/ABS = \varphi_{P_0}$	0.81 (1)	0.70 (0.87)	0.79 (0.98)
$ET_0/TR_0 = \psi_0$	0.44 (1)	0.30 (0.69)	0.43 (0.98)
$ET_0/ABS = \varphi_{E_0}$	0.35 (1)	0.21 (0.60)	0.34 (0.96)
<b>DENSITY OF RCs</b>			
$RC/CS_0$	345 (1)	218 (0.63)	278 (0.81)
$RC/CS_M$	1797 (1)	726 (0.40)	1347 (0.75)
<b>FLUXES - ACTIVITIES per CS</b>			
$ABS/CS_0$	620 (1)	610 (0.98)	612 (0.99)
$TR_0/CS_0$	501 (1)	427 (0.85)	486 (0.97)
$ET_0/CS_0$	218 (1)	128 (0.59)	208 (0.95)
$ABS/CS_M$	3229 (1)	2033 (0.63)	2963 (0.92)
$TR_0/CS_M$	2609 (1)	1423 (0.55)	2351 (0.90)
$ET_0/CS_M$	1137 (1)	428 (0.38)	1007 (0.89)
<b>PERFORMANCE INDEX and components</b>			
$(RC/ABS)$	0.56 (1)	0.36 (0.64)	0.46 (0.82)
$\varphi_{P_0} / (1 - \varphi_{P_0})$	4.21 (1)	2.33 (0.55)	3.84 (0.91)
$\psi_0 / (1 - \psi_0)$	0.77 (1)	0.43 (0.56)	0.75 (0.97)
$PI_{ABS}$	1.81 (1)	0.36 (0.20)	1.31 (0.72)

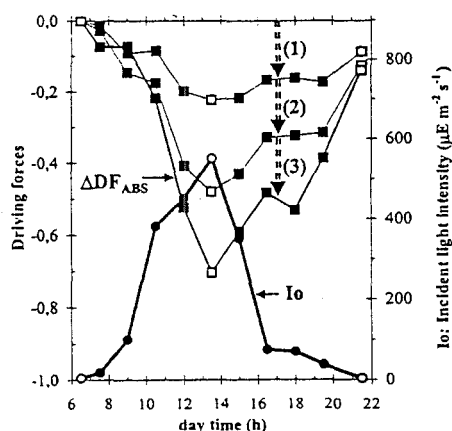
Table 3. The values of the different structural and functional parameters provided by the JIP-test, calculated from the data of Table 2. Numbers in parentheses express the normalized values over the corresponding values at 6:30 pm (control)

over the corresponding values at 6:30, to facilitate the comparison.

Various PSII behaviour patterns can be constructed from the values of the calculated parameters. We here chose to present the changes of the PSII behaviour during the diurnal changes, by means of the driving forces, expressed by their difference,  $\Delta DF$ , from the corresponding values at 6:30 (Figure 3). As above explained, the logarithm of the performance index, defined as the driving force for photosynthesis,  $DF_{ABS} = \log[PI_{ABS}]$ , can be written as the sum of three partial driving forces. Similarly, we get  $\Delta DF_{ABS} = \Delta DF_{RC} + \Delta DF_{\phi} + \Delta DF_{\psi}$ . Figure 3 shows, not only the time course of  $\Delta DF_{ABS}$ , but also how it is built up: deviation from zero marked by the arrow (1) is due to the  $\Delta DF_{RC}$  term; the further deviation, marked by the arrow (2) is due to the  $\Delta DF_{\phi}$  term; the last deviation, marked by the arrow (3), comes from the  $\Delta DF_{\psi}$  term and leads to the driving force difference,  $\Delta DF_{ABS}$ . For comparison, the incident light intensity ( $I_0$ ) vs. daytime is presented in the same figure.

As clearly demonstrated in Figure 3, the JIP-test offers the possibility to analyse in details the mechanistics of the variations in the driving forces. For many stress responses we observed, like in Figure 3, that a fraction of the reaction centres of PS II are inactivated by being converted to non  $Q_A$  reducing centres (see e.g. Srivastava and Strasser, 1999; Srivastava et al., 1999; Tsimilli-Michael, 1999; 2000). Thus, these centres become "silent RCs" in respect to photochemistry (Strasser et al. 2000) and dissipate as heat the trapped excitation energy, i.e. they act as heat radiators, hence called quenching or heat sinks. However, they are again turned on as soon as "cooling" of the plant is no more needed. This type of regulation corresponds to a digital switching between two states of the RCs, namely the  $Q_A$  reducing (fully active energy conserving units) and the  $Q_A$  non reducing (silent, heat dissipating units).

Figure 3. The total driving force difference  $\Delta DF_{ABS} = \Delta DF_{RC} + \Delta DF_{\phi} + \Delta DF_{\psi}$ , where the values at 6:30 were used as reference, vs. the day-time, along with the incident light intensity ( $I_0$ ). The figure shows also how this total driving force is built up: deviation



from zero marked by the arrow (1) is due to the  $\Delta DF_{RC}$  term; the further deviation, marked by the arrow (2) is due to the  $\Delta DF_{\phi}$  term; the last deviation, marked by the arrow (3), comes from the  $\Delta DF_{\psi}$  term and leads to the total driving force difference,  $\Delta DF_{ABS}$ . Open points refer to the measurements at 6:30, 13:30 and 21:30, for which the full sets of parameters are presented in Table 3

## Concluding Remarks

In this overview we have discussed how the chlorophyll *a* fluorescence induction curves can be utilised to derive information about the behaviour of PSII and thus to detect, describe and quantify stress effects. However, the recorded fluorescence transients carry much more information than those used by the JIP-test. The whole of this information can be utilized by means of the powerful methods of numerical simulations (see e.g. Stirbet et al., 1998). The application of these methods is being further explored in our laboratory.

The fluorescence kinetic is so rich in information that since its discovery in 1931 new properties are continuously being detected. New instrumentation has made it possible to measure fast changes in a way that one can follow the electron transfer from the water splitting side to  $Q_A$ , then to  $Q_B$  and later to plastoquinone. As this transfer is very sensitive to stressors and highly dependent on the need of electrons for metabolism, PSII fluorescence can become a biosensing device for stress detection in plants. For such a detection, the establishment and application of JIP-test which can screen many samples in a short time is very useful. The capabilities of the JIP-test for stress diagnosis are now well understood and accepted, as e.g. presented recently by Baillod and Martini (2001) in the Biotechnology Journal "Bioworld". At a second stage, more time consuming and specific tests can be made on selected samples. In the future, the newer fluorescence imaging techniques and numerical simulations will have to be calibrated by accurate kinetic tests like the JIP-test.

Basic fluorescence understanding combined with the JIP-test are a tool to analyse any plant material in any situation, even by non fluorescence specialist. We hope that many young scientists will try the JIP-test presented in this report, to probe photosynthesis.

## Footnote

\* Dedicated to Professor Dr. C. Sironval who stimulated me (R.J. Strasser), already 30 years ago, to work in this area and to give a communication (Strasser R.J., 1973, Induction phenomena in green plants when the photosynthetic apparatus starts to work. Arch Internat Physiol Biochim 81: 935-955) at the very same place that I gave recently the communication presented here in this publication.

## References

- Baillod P.M. & Martini G.O., 2001, Towards an optoelectronic future: Photosynthetic fluorescence enables simple environmental assays. *Bioworld* (2), 14-16 (ISSN 1424-8514).
- Clark A.J., Landolt W., Bucher J. & Strasser R.J., 1998, The response of *Fagus sylvatica* to elevated  $CO_2$  and ozone probed by the JIP-test based on the chlorophyll fluorescence rise OJIP. In: L.J. De Kok & I. Stulen, eds. *Responses of Plant Metabolism to Air Pollution and Global Change*, Backhuys Publishers, Leiden, The Netherlands, pp 283-286.
- Clark A.J., Landolt W., Bucher J. & Strasser R.J., 2000, Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll *a* fluorescence performance index. *Environmental Pollution* 109: 501-507.
- Govindjee, 1995, Sixty-three years since Kautsky: Chlorophyll *a* fluorescence. *Aust J Plant Physiol* 22: 131-160.
- Krause G.H. & Weiss E., 1991, Chlorophyll fluorescence and photosynthesis:

the basics. *Annu Rev Plant Physiol Plant Mol Biol* 42: 313-349.

- Larcher W., 1987, Stress bei Pflanzen. *Naturwissenschaften* 74: 158-167.
- Krüger G.H.J., Tsimilli-Michael M. & Strasser R.J., 1997, Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. *Physiol Plant* 101: 265-277.
- Ouzounidou G., Moustakas M. & Strasser R.J., 1997, Sites of action of copper in the photosynthetic apparatus of maize leaves: Kinetic analysis of chlorophyll fluorescence, oxygen evolution, absorption changes and thermal dissipation as monitored by photoacoustic signals. *Aust J Plant Physiol* 24: 81-90.
- Srivastava A. & Strasser R.J., 1996, Stress and stress management of land plants during a regular day. *J Plant Physiol* 148: 445-455.
- Srivastava A. & Strasser R.J., 1997, Constructive and destructive actions of light on the photosynthetic apparatus. *J Sci Ind Res* 56: 133-148.
- Srivastava A. & Strasser R.J., 1999, Survival Strategies of Plant to Cope the Stress of Daily Atmospheric Changes. In: *Crop Improvement for Food Security*, R.K. Behl, M.S. Punia & B.P.S. Lather, eds. SSARM, Hisar, India, ISBN 81-87267-01-1, Chapter 6, pp. 60-71.
- Srivastava A., Strasser R.J. & Govindjee, 1999, Greening of peas: parallel measurements of 77K emission spectra, OJIP chlorophyll a fluorescence transient, period four oscillation of the initial fluorescence level, delayed light emission, and P700\*. *Photosynthetica* 37: 365-392.
- Stirbet A., Govindjee, Strasser B.J. & Strasser R.J., 1998, Chlorophyll a fluorescence induction in higher plants: modelling and numerical simulation. *J Theor Bio.* 193: 131-151.
- Strasser B.J. & Strasser R.J., 1995, Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: P. Mathis, ed. *Photosynthesis: From Light to Biosphere*, Kluwer Academic Publishers, The Netherlands, Vol V, pp 977-980.
- Strasser R.J., 1985, Dissipative Strukturen als Thermodynamischer Regelkreis des Photosynthese-apparates. *Ber Deutsche Bot Ges Bd* 98: 53-72.
- Strasser R.J., 1988, A concept for stress and its application in remote sensing. In: H.K. Lichtenthaler, ed. *Applications of Chlorophyll Fluorescence*, Kluwer Academic Publishers, The Netherlands, pp 333-336.
- Strasser R.J. & Govindjee, 1992, The Fo and the O-J-I-P fluorescence rise in higher plants and algae. In: J.H. Argyroudi-Akoyunoglou, ed. *Regulation of Chloroplast Biogenesis*, Plenum Press, New York, pp 423-426.
- Strasser R.J., Srivastava A. & Govindjee, 1995, Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem Photobiol* 61: 32-42.
- Strasser R.J., Srivastava A. & Tsimilli-Michael M., 1999, Screening the Vitality and Photosynthetic Activity of Plants by the Fluorescence Transient. In: *Crop Improvement for Food Security*, R.K. Behl, M.S. Punia & B.P.S. Lather, eds. SSARM, Hisar, India, ISBN 81-87267-01-1, Chapter 7, pp 72-115.
- Strasser R.J., Srivastava A. & Tsimilli-Michael M., 2000, The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: M. Yunus, U. Pathre & P. Mohanty, eds. *Probing Photosynthesis: Mechanism, Regulation and Adaptation*, Taylor and Francis, London, UK, ISBN 0-748-40821-5, Chapter 25, pp 443-480.
- Tsimilli-Michael M. & Strasser R.J., 2001, Mycorrhization as a stress adaptation procedure. In: S. Gianinazzi, H. Schüepp & K. Haselwandter, eds. *Mycorrhizal Technology in Agriculture from Genes to Bioproducts*, Birkhauser (in press).
- Tsimilli-Michael M., Krüger G.H.J. & Strasser R.J., 1996, About the perpetual state changes in plants approaching harmony with their environment. *Archs Sci Genève* 49: 173-203.
- Tsimilli-Michael M., Pêcheux M., & Strasser R.J., 1999, Light and heat stress adaptation of the symbionts of temperate and coral reef foraminifers probed in hospite by the chlorophyll a fluorescence kinetics O-J-I-P. *Z Naturforsch* 54C: 671-680.
- Tsimilli-Michael M., Eggenberg P., Biro B., Köves-Pechy K., Vörös I. & Strasser R.J., 2000, Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and Azospirillum and Rhizobium nitrogen-fixers on the photosynthetic activity of alfalfa, probed by the chlorophyll a polyphasic fluorescence transient O-J-I-P. *Applied Soil Ecology* 15: 169-182.
- Van Rensburg L., Krüger G.H.J., Eggenberg P. & Strasser, R.J., 1996, Can screening criteria for drought resistance in *Nicotiana tabacum* L. be derived from the polyphasic rise of the chlorophyll a fluorescence transient (OJIP)?

Division Chimie médicinale

Rubrique de la SRC



## European Federation for Medicinal Chemistry (EFMC) Awards: Call for Nominations

### NAUTA AWARD ON PHARMACOCHEMISTRY

The Nauta Award has been established to honour the memory of Prof. Dr. W. Th. Nauta, whose activities have been very important for the advancement of Medicinal Chemistry in general, and for the development of international organisational structures for this discipline. The Award consisted of a diploma and 7500€, and is given for outstanding of Medicinal Chemistry to a scientist working in Europe or to a European scientist abroad. In exceptional cases the Award will acknowledge contributions fostering the cooperation among medicinal chemists in Europe.

The Award will be conferred for the sixth time on the occasion of the XVIIth International Symposium on Medicinal Chemistry in Barcelona, Spain (September 1-5, 2002), during which the recipient will be invited to give presentation. Previous recipients were: Dr. A.E. Brändström 1992, Dr. M. Petitou 1994, Prof. Dr. P. Krogsgaard-Larsen 1996, Prof. Dr. H. Timmerman 1998, and Prof. Dr. E. De Clercq 2000.

### UCB AWARD FOR EXCELLENCE IN MEDICINAL CHEMISTRY

UCB S.A. and EFMC are pleased to announce the creation of a new Award, the "UCB Award for Excellence in Medicinal Chemistry". The Award will consist of a diploma, 7.500 € and an invitation for a lecture by the Award recipient. The Award will be given biennially, starting at the XVIIth International Symposium on Medicinal Chemistry, in Barcelona, Spain (September 1-5, 2002), for which the recipient will be invited to lecture.

This Award, established by UCB S.A., Pharma Sector, will acknowledge and recognise outstanding scientific research, without restrictions regarding nationality, in the field of Medicinal Chemistry in its broadest sense.

**Nominations:** Nominations for the Sixth Nauta Award, and for the First UCB Award should be submitted to the Chairman of the Juries, Prof. Dr. Henk Timmerman, EFMC President, Vrije Universiteit LACDR, Department of Pharmacocchemistry, De Boelelaan 1083, Postbus 7161, NL-1081 HV Amsterdam, The Netherlands, Fax +31 20 444 76 10,

E-mail: timmermn@chem.vu.nl, not later than December 31, 2001. The proposals should include a short curriculum vitae, a list of publications and indicate the reasons for which the candidate qualifies for the Award. Details of the regulations can be found at the EFMC web site ([www.efmc.ch](http://www.efmc.ch)).